REMARKS

Claims 174 and 176 are currently pending.

These claims stand rejected under 35 USC §112, firs paragraph, as failing to comply with the written description requirement for the reasons of record set forth in previous office actions. It has been maintained that "the specification does not describe which portions of the sequence encoding the desaturase would function in cosuppressing expression of the endogenous gene. . ."

As was stated in the previous response dated November 12, 2004:

Those "skilled in the art know that an entire coding region is not needed to down-regulate expression of a gene. Submitted herewith is a copy U.S. Patent No. 5,231,020 issued to Jorgensen et al. on July 27, 1993) (copy enclosed) supporting this very aspect. Attention is kindly invited to column 8 at lines 1-19 which states, *inter alia*, that '. . . It should be noted that since a full length coding sequence is unnecessary, it is possible to produce the same effect on multiple proteins together to coordinately repress various different genes." It is stated in column 8 at lines 9-14 that a "sequence of greater than 50-100 nucleotides should be used, though a sequence of greater than about 200-300 nucleotides would be preferred, and a sequence of greater than 500-1000 nucleotides would be especially preferred depending on the size of the endogenous gene. ' All of this is information available to those of ordinary skill in the art."

Thus, it is respectfully submitted that the specification does describe that all or part of the sequence encoding a desaturase can be used in down-regulating expression whether the sequence is part of the coding region or is part of a non-coding region.

Withdrawal of this ground of rejection is respectfully requested.

Claims 174 and 176 stand rejected under 35 USC §112, first paragraph, as failing to comply with the enablement requirement. A concern was raised on page 2 of the Office Action that stating that "'part of the shrunken-1 intron'" was used, does not teach the specific sequence.

As was stated in the previous response dated November 12, 2004:

"pBN431 is a derivative of plasmid vectors pBN427 (Fig. 7A) and pBN414 (Fig. 3E) and was assembled from one of the purified HIND III digest products obtained from each of these two plasmids. "

Specifically, Examples 6 through 8 are believed to set forth the pertinent information:

a) pBN414 was made as set forth on page 42 at lines 21-26 of the specification:

> The 0.9 kb Nco I fragment of the delta-9 desaturase gene**(SEQ ID NO:8)** was also cloned into the Nco I site of **pBN257** to create a construct, **pBN414**, containing a fused trait gene of fad2-1 and delta-9desaturase, both in the sense orientation, **as shown in Figure 3E.** The coding sequence of fad2-1 in pBN414 is out of frame as in pBN257, and its C-terminal sequence was interrupted by the insertion of the delta-9desaturase fragment (79% of the full length coding region shown in SEQ ID NO:8).

b) pBN257 was made from **pBN256** which was made from **pBN220.**

1) Plasmid pBN220 (construct) is set forth on page 40 in Table 2.

pBN220 comprises **SEQ ID NO:39** which is the 0.9 kb oleosin promoter. The 0.9kb oleosin promoter corresponds to **nucleotides 749-1619 of (SEQ ID NO:1) as is set forth in Table 1 on page 37 at line 6.** It is stated on page 41 at lines 7-15 of the specification that:

> Intron enhancement is very important in optimizing gene expression. None of the constructs lacking the Sh1 element provided any significant level of GUS expression in the assay. The oleosin 16 kDa promoter with an optimized length and composition, as in pBN220, was found to be stronger than the globulin-1 promoter (as contained in pSM100). The results of the Northern blot analyses characterizing early timing of expression in the young developing corn embryos, combined with the demonstration of its high activity in the expression assay, indicated that **the optimal embryo/aleurone-specific promoter is the 0.9 kb fragment (SEQ ID NO:39) isolated from the oleosin 16 kDa gene combined with a Sh1 exon 1/intron 1 element in the 5'-untranslated region.** (Emphasis added.)

It is stated on page 36 at lines 36-38 of the specification that the **exon 1/intron 1 fragment corresponding to nucleotide position 1138-2220 in accession number X02382,Gen Bank, of maize shrunken-1 gene was cloned into the 5'untranslated region** as described in Example 6 on pages 36-41 of the specification. (See also note (d) on page 38 at lines 5-8 of the specification).

Accordingly, it is respectfully submitted that the "part of the shrunken-1 intron" used is indeed sufficiently described.

2) The construction of pBN220 was described above. It is stated on page 41 of the specification at lines 28-33 that:

> An intermediate construct, **pBN256, modified from pBN220** was made as the starting vector for the various expression constructs with lipid trait genes. pBN220 was digested with NcoI and EcoRI to delete the GUS coding sequence, end-filled with dNTPs and Klenow fragment of DNA polymerase I, and re-ligated. The resulting plasmid was designated pBN256 (Figure 3A).
>
> PCR was used to obtain a fragment containing the fad2-1 coding region with Kpn I restriction site at both ends. The fad2-1 cDNA clone was used as the template with primers (SEQ ID NOS:50 and 51) specific to the fad2-1 sequence each containing a site for KpnI (underlined).
> 5'- CGGGGTACCGATGACCGAGAAGGAGCGGG-3' SEQ ID NOS:50
> 5'-GGCGGTACCTAGAACTTCTTGTTGTACCA-3' SEQ ID NOS:51
>
> The expected 1.2 kb fragment was gel-purified, digested with Kpn I, and cloned into a vector with a comparative Kpn I site to facilitate propogation and further manipulation. **The Kpn I fragment was digested out from this new construct, and the ends were blunted as above, inserted into the Sma I site of pBN256, to become pBN257.** This clone contains a near full-length of fad2-1 coding region, but the ATG translation start codon is out of frame (Figure 3B).
>
> A DNA fragment containing the delta-9 desaturase coding region was recovered by PCR using the delta-9 desaturase cDNA clone (SEQ ID NO:8) DNA as the template and coding region-specific primers (SEQ ID NOS:52 and 53) that contained NcoI sites. The resulting fragment was gel purified, cut by Nco I, and inserted into the Nco I site of the modified pBN220 in which the GUS gene had been previously removed.

5'-GGCCTCCGCCATGGCGCTCCGCTCCACGACG-3' SEQ ID NOS:52
5'-CTCCAACTCAAGCAGTCGCCATGGGTTTCC-3') SEQ ID NOS:53

> (Plasmid pBN220 was cut by Nco I and Sma I to remove the GUS gene, end-filled in by Klenow treatment, and religated as the modified GUS-free vector.) The resulting clones contained a truncated corn delta-9 desaturase coding region (approximately 0.9 kb, comprising 79% of the full-length coding sequence) in each of the two possible orientations, sense (pBN264, Figure 3C) and antisense (pBN262, Figure 3D).
>
> **The 0.9 kb Nco I fragment of the delta-9 desaturase gene(SEQ ID NO:8) was also cloned into the Nco I site of pBN257 to create a**

construct, pBN414, containing a fused trait gene of fad2-1 and delta-9desaturase, both in the sense orientation, as shown in Figure 3E. The coding sequence of fad2-1 in pBN414 is out of frame as in pBN257, and its C-terminal sequence was interrupted by the insertion of the delta-9desaturase fragment (79% of the full length coding region shown in SEQ ID NO:8).

b) Plasmid 427 is described on page 46 at lines 14-26 of the specification.

Using processes similar to those described above, new transgenic events with high stearate – and hence high saturate - phenotypes were generated (Table 4). The trait gene constructs used in these experiments are from either pBN264 or pBN427 (Figure 7A). **Plasmid pBN264 is similar to the pBN262, except that the delta-9 desaturase is in a sense orientation relative to the promoter. The transgene sequence is contained within a Sal I fragment (position 3248-44) of pBN427 and is identical to the corresponding Sal I fragment of pBN264 (position 2-3206).** However, **pBN427 uses a vector backbone with a hygromycin resistance selectable marker (HPT, from pKS17, described in WO 94/11516),** versus the ampicillin marker in pBN262 and pBN264. The transgene prepared for the bombardment were either the restriction enzyme digested and agarose gel purified DNA fragment from pBN264 (for events derived from the FA025 experiment, the transgene fragment was marked as fBN264), or the intact pBN427 plasmid DNA (for events derived from the FA029 experiment). The restriction enzyme used to cut out the transgene may be Sal I or Xba I, which release a transcriptionally functional transgene fragment of 3.2 kb, which can then be purified following agarose gel electrophoresis. The use of a transgene DNA fragment, rather than the entire plasmid, allows the recovery of transgenic events which do not contain a bacterial antibiotic resistance gene.

pBN262 and pBN264 are described in Example 7 and were made from pBN220 (which is discussed above):

(Plasmid pBN220 was cut by Nco I and Sma I to remove the GUS gene, end-filled in by Klenow treatment, and religated as the modified GUS-free vector.) **The resulting clones contained a truncated corn delta-9desaturase coding region (approximately 0.9 kb, comprising 79% of the full-length coding sequence) in each of the two possible orientations, sense (pBN264, Figure 3C) and antisense (pBN262, Figure 3D).**

It is respectfully submitted that the particular sequence used is sufficiently described in the specification for all of the reasons set forth above.

Even though Applicants believe that pBN431 is sufficiently described in the specification, in an abundance of caution, a deposit of plasmid pBN431 was made to facilitate prosecution. A copy of the deposit receipt for pBN431 from the ATCC accompanies this Response After Final.

A petition for a one (1) month Extension of Time accompanies this Response After Final along with a copy of the ATCC deposit receipt for pBN431.

A Notice of Appeal also accompanies these papers.

In view of the foregoing, it is respectfully submitted that the claims are now in form for allowance which allowance is respectfully solicited.

Please charge any requisite fees associated with the filing of this response including, but not limited to, the Petition for Extension of time for one (1) month or credit any overpayment to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Respectfully submitted,

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